Nanogen, Inc.

Microchip To Speed DNA Analysis Process

Whether or not a patient will respond well to a particular drug is dependent on his or her genetic composition. In 1995, however, there were significant barriers to performing diagnostic tests using DNA samples. Machines capable of DNA diagnostic work were large, expensive, cumbersome, and in short supply. Moreover, they required lengthy sample preparation work before the diagnostic process could even begin. This dearth of available, cost-effective processes often prevented the use of DNA sequencing in diagnostic work. Given the promise of DNA-based diagnostics, industry leaders indicated that the question was not whether diagnostic work would adopt a DNA-based approach, but when the adoption would begin. The Advanced Technology Program (ATP) hoped to accelerate that shift through a focused competition entitled "Tools for DNA Diagnostics." Nanogen responded by submitting a proposal to develop a microlaboratory (a diagnostic lab on a microchip) for DNA diagnostics that would reduce the previously cumbersome sample preparation steps for DNA to a single, rapid process carried out on a single microchip.

In August 1995, ATP awarded Nanogen funds to pursue the development of its DNA diagnostic sample preparation system. Nanogen successfully reduced sample preparation time significantly and created a microlaboratory, which represented the first step towards making DNA diagnostics affordable and effective. Through the assistance of a separate ATP grant, Nanogen narrowed the microlaboratory's focus and reduced the size and cost of the machine in order to commercialize a product. As of February 2003, Nanogen reported product revenues of $3.4 million, driven by two staple products, the NanoChip® workstation and the NanoChip® microarray.

COMPOSITE PERFORMANCE SCORE
(based on a four star rating)
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Research and data for Status Report 95-08-0009 were collected during June 2001 - December 2002, and February 2003.

Potential To Harness the Human Genome Project for Improved Healthcare

Since 1990, the National Institutes of Health and the Department of Energy have been funding the Human Genome Project to sequence and map out the thousands of individual genes strung along the 46 chromosomes in human cells. The human genome map was expected to provide a multitude of insights into poorly understood diseases and biological phenomena, as well as new treatments for genetically based ailments. DNA diagnostics would link the information learned from the gene map to personalized medical treatments for each patient.

As the Human Genome Project opened up new horizons and possibilities for the diagnosis and treatment of diseases, scientists needed fast, reliable diagnostic tools that were inexpensive enough to fit into healthcare budgets. To that end, ATP took a lead role in funding the technology's development. In 1992, the in vitro portion of DNA diagnostics (tests performed outside the patient's body) had a market size of $58 million. The entire diagnostics market was $5 billion. ATP funding helped in vitro DNA diagnostics to become a more mature, competitive industry with many new products. In fact, by 1997, the market size for diagnostics was $20 billion, with in vitro DNA diagnostics accounting for more than $500 million of that amount.

While the potential for growth in the DNA diagnostics market was predictable, it was not clear exactly what apparatuses would come to market and fuel that growth. Nanogen's plan to use electric current to attract DNA to the capture probes had the potential to speed up the process of sample preparation, making DNA diagnostic work faster and less expensive.

In 1995, there were significant barriers to performing diagnostic tests using DNA samples.

Nanogen's proposal to ATP represented a critical step in developing an enabling technology to use the knowledge gained from the Human Genome Project to generate better, cheaper, and more accurate DNA diagnostic work. In 1995, ATP awarded Nanogen two years of cost-shared funds totaling $2 million to give the company a chance to advance its promising technology plan.

Existing Diagnostic Methods Are Slow and Costly

In 1995, DNA diagnostic work required significant sample preparation before the process of analyzing the code and diagnosing ailments could begin. The preparation process typically involved cutting the strand with particular enzymes and running the strands through a gel using electric current. Since DNA itself carries a negative charge, it follows the current and works its way from the negative end of the gel to the positive end of the gel. Longer strands take longer to work their way through the gel molecules; therefore, the DNA separates by size into a predictable pattern. The particular portion of interest within particular strands then had to be separated and prepared for analysis. The strands with the appropriate genes could then be compared against other like strands to test for defects.

In 1995, there were products on the market that could run 96 gels in parallel, separate the DNA in 90 minutes, and use a computer to read the results directly from the gels. Older technology still on the market took up to six hours for the DNA to separate in the gel. Each of these technologies, however, required significant preparation work to further separate and amplify particular DNA segments.

Microchip and Biological Advances Could Slash Sample Preparation Time

Nanogen proposed to develop an integrated system of microelectronic components that rely on electronically controlled properties of cells and molecules to achieve separation, selectivity, amplification, and identification of medically relevant DNA sequences. In the proposed Nanogen process, a sample (usually a drop of blood) would be placed onto the chip. A cell selector site would then exploit the different binding affinities of different cell types under different electric conditions to select the appropriate cells for analysis.

The next site on the chip would rely on the same concept of binding affinity to separate and extract relevant DNA from the other components of the selected cells. Yet another site would pass the DNA over an array of probes designed to locate, bond with, and identify genetic material associated with defects or infectious diseases. Finally, each selected sample fragment would be analyzed in detail for specific genetic mutations or patterns. If any specific defects were found, a dye would bind to that location. From the pattern of colors on the chip, diagnostic scientists could potentially infer the sample DNA's sequence and diagnose conditions or prescribe treatment from that sequence.

A computer capable of reading and understanding the pattern of dyes on diagnostic chips would be required for Nanogen's innovation to become commercially viable. Nanogen proposed to create a microelectronic DNA diagnostic system contained on one microchip. If successful, the sample preparation step in DNA diagnostics would be eliminated, making the process significantly less time-consuming.

Nanogen Explores Potential of Enabling Technology

Nanogen's proposal to create a diagnostic lab on a microchip held the potential to develop the enabling technology of rapid sample preparation for DNA-based diagnostics. While other technological advances would be necessary to analyze the results of sample preparation in order for affordable DNA-based diagnostics to come to market, Nanogen's research plan would enable those other technological advances by setting a common standard for sample preparation. If the project succeeded, developers of sample analysis machines would be able to start product development from a defined standard for samples and work toward a universal reader for DNA diagnostics. This would save time and money throughout the entire DNA diagnostics industry.

Nanogen proposed to create a microelectronic DNA diagnostic system contained on one microchip.

ATP funds were necessary to support this research because Nanogen proposed a high-risk use of electricity, chemistry, and microfluidics that might not succeed. As a start-up company, Nanogen could not afford to fund the development of an integrated system for DNA diagnostic machines; instead, it focused most of its spending on supporting its existing products. Even if it did succeed, Nanogen could not reap all the economic benefits of the standard they would set for the DNA diagnostic industry. A successful project could enable spillover effects across many economic sectors by combining the knowledge from the Human Genome Project with innovations in microfluidics and computing power to create an entirely new industry.

Nanogen Solves DNA Diagnostic System Technical Issues

Because Nanogen planned to create an entirely new diagnostic industry, the company realized early that it had to build a new type of DNA diagnostic machine from the ground up. With ATP funding, Nanogen sought to merge a microchip, electric current, waveguides, and low-power lasers into an integrated microelectronic DNA diagnostic system. Nanogen had to overcome three major technical problems in order to achieve success.

The first, overarching technical challenge for Nanogen scientists was to create a microchip that could reduce or eliminate sample preparation times. Taken separately, the components of this chip were not remarkable. Integrating knowledge and applications from a diverse array of industries and enabling them to operate on a high throughput chip was a remarkably risky technical endeavor. By circumventing or solving technical obstacles as they arose throughout the course of the ATP-funded project, Nanogen achieved its ultimate goal of reducing sample preparation times from hours to minutes. Moreover, much of the sample preparation work was automated, freeing researchers to do other research rather than work on time-consuming sample preparation.

The second technical challenge was to find a way to fit a waveguide small enough to focus a low-power laser onto the DNA diagnostic chip so that it could accurately read the data, but not destroy the sample. Electric current had not been used to separate DNA prior to the Nanogen project. Lasers and waveguides available at the time of the ATP-funded project would have sent too much current through the sample, essentially electrocuting the biological sample. To solve this problem, Nanogen experimented with different types of waveguides and laser frequencies used in other industries until scientists found a combination that functioned properly and did not destroy the sample. The resulting waveguide was a new application for existing technology.

The third technical challenge, addressed toward the end of the ATP-funded project, involved expanding the screening ability of Nanogen's microchip. The early prototype diagnostic chip was incapable of screening DNA segments with more than just a couple of genetic defects. Screening for a large number of defects is critically important for a DNA diagnostic system because genetic abnormalities often result from four or five defects on a gene, rather than just one. Each defect must be located, replicated and amplified with a polymerase chain reaction, marked with dye, and then run through a diagnostic machine. The early diagnostic chip's prototype camera became fooled by a large number of dye-marked defects and recorded interference rather than an accurate picture of genetic abnormalities.

In the final year of the ATP-funded project, Nanogen succeeded in implanting better capture probes using improved chemistry and electronic circuitry for active hybridization. The result was that Nanogen scientists "multiplexed" four areas on the chip and partitioned it in a way that prevented interference.

Nanogen reduced the time needed for the entire process, from preparation through amplification to analysis, from hours to minutes and eliminated the extensive sample preparation work.

Conclusion

In 1995, Nanogen scientists devised a research plan to create the generic technology for a DNA diagnostic microlaboratory and received funding from the ATP focused program "Tools for DNA Diagnostics." The project's objective was to combine previously cumbersome sample preparation steps with an analysis chip that could substantially reduce the time and expense of analyzing DNA. Nanogen succeeded in combining the sample preparation and analysis steps into one process that took minutes instead of hours and freed up scientists to focus on other research instead of preparation work. Nanogen developed a DNA microlaboratory through additional research after the close of this ATP-funded research. As of February 2003, the company is generating annual product revenues of $3.4 million from sales of its NanoChip® array and a workstation.

PROJECT HIGHLIGHTS
Nanogen, Inc.

Project Title: Microchip To Speed DNA Analysis Process (An Integrated Microelectronic DNA Diagnostic System)

Project: To speed the entry of cost-effective DNA analysis into the clinical diagnostic laboratory through the development of an integrated system to carry out all necessary sample preparation and analytical procedures in a linked series of microfabricated sites that sum into a microlaboratory on a single chip.

Duration: 8/1/1995-12/31/1997
ATP Number: 95-08-0009

Funding (in thousands):

ATP Final Cost	$2,000	57%
Participant Final Cost	1,500	43%
Total	$3,500	

Accomplishments: Nanogen developed a microchip-sized DNA preparation system that uses electric current to actively hybridize DNA, allowing for faster and less expensive DNA diagnostic work. The highlights of this technology, and its spillover effects, include:

- Development of a small DNA diagnostic chip that quickly and effectively prepares DNA and conducts diagnostic analysis in minutes instead of hours.
- Incorporation of waveguides and low-powered laser light into a microchip to create an active hybridization process that allows DNA to be prepared for analysis significantly faster than before. By automating the sample preparation process, Nanogen freed researchers to conduct research rather than prepare samples.
- Publication of an article written by Nanogen scientists in the April 2000 issue of Nucleic Acids Research entitled, "Rapid, High Fidelity Analysis of Simple Sequence Repeats on an Electronically Active DNA Microchip."

Nanogen applied for three patents (two were granted), with the accompanying disclosure of technical innovations as part of the patent application process. The patents Nanogen received from this project are:

- "Apparatus and methods for active biological sample preparation"
  (No. 6,129,828: filed September 6, 1996, granted October 10, 2000)
- "Channel-less separation of bioparticles on a bioelectronic chip by dielectrophoresis"
  (No. 6,071,394: filed January 30, 1998, granted June 6, 2000)

Commercialization Status: Nanogen is continuing research into developing the necessary computer system, optical-imaging capabilities, and databases of genetic sequences that would enable commercialization of the portable genetic analysis system. As of February 2003, Nanogen was generating $3.4 million in product revenues per year from products such as the NanoChip® array and the workstation.

Outlook: The market for DNA diagnostic machines is expected to increase substantially if other technologies become available to harness the power of Nanogen's innovation. Until those technologies appear, however, the outlook is uncertain.

Composite Performance Score: * *

Number of Employees: 48 employees at project start, 160 as of December 2002

Focused Program: Tools for DNA Diagnostics, 1995

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